Effect of *Aloe vera* Leaf Gel and Pulp Extracts on the Liver in Type-II Diabetic Rat Models

Ayse CAN, a Nuriye AKEV, a, b Nurten OZSOY, a Sehnaz BOLKENT, b Bahriye Pelin ARDA, b Refiye YANARDAG, c and Alper OKYAR d

a Department of Biochemistry, Faculty of Pharmacy, Istanbul University; 34116-Universite, Istanbul, Turkey; b Department of Biology, Faculty of Science, Istanbul University; 34134-Vezneciler, Istanbul, Turkey; c Department of Chemistry, Faculty of Engineering, Istanbul University; 34320-Avcilar, Istanbul, Turkey; and d Department of Pharmacology, Faculty of Pharmacy, Istanbul University; 34116-Universite, Istanbul, Turkey.

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Diabetes is recognised as one of the leading causes of morbidity and mortality in the world. About 2.5—3% of the world’s population suffers from this disease, a proportion which, in some countries, can reach 7% or more. Hyperglycemia leads to metabolic disorders and various complications. 1)

There is increasing evidence indicating that enhanced production of free radicals may be an important contributing factor in the complications seen in diabetes. Species increased in diabetes, especially in uncontrolled diabetes, can lead to the autooxidation of glycosylated proteins, activation of the sorbitol pathway, induction of membrane damage, and oxidation of cellular lipids and proteins. 2)

Many traditional plant treatments for diabetes mellitus are used throughout the world. 3) Management of diabetes without any side effects is still a challenge to the medical system. This leads to increasing demand for natural products with antidiabetic activity and fewer side effects. 4) Many herbs and plant products have been shown to have hypoglycemic action. 5) *Aloe vera* (L.) B. URM. fil. (synonym *A. barbadensis Miller*) (Liliaceae), native in North Africa, is one of these antidiabetic plants. *Aloe* species have been used for centuries for their various healing properties. 6) In the last decade there have also been reports on the antidiabetic activity of *Aloe* extracts. 7, 8)

The *Aloe* plant contains anthraquinone glycosides, especially in the latex which is different from the gel, polysaccharides, aloesins, glycomannans and β-sitosterol. 9, 10) Antioxidative phenolic compounds were recently isolated from *Aloe barbadensis* and identified as aloesin derivatives. 11, 12)

In our previous studies we have reported the blood glucose lowering effect of *Aloe vera* leaf pulp and gel extracts on neonatal streptozotocin (n0STZ)-induced type-II diabetic rats in acute 13) and chronic 14) treatment. It is well known that STZ causes diabetes by the selective degeneration of pancreatic β-cells. To date, research on traditional antidiabetic plants has been especially focused on STZ-induced type-I diabetic rats. It is assumed that herbal medicine can only be effective as an alternative to oral hypoglycemic agents, in type-II diabetes where pancreatic islets are not totally destroyed. That is why, n0STZ-induced type-II diabetic rats 15) were used in our investigation as well as glibenclamide, a known hypoglycemic agent, for comparison.

The present study was carried out to evaluate whether *Aloe* leaf pulp and gel extracts had any protective or harmful effect on the liver injury caused by type-II diabetes in n0STZ-induced diabetic rats.

**Materials and Methods**

**Plant Material** Specimens of *Aloe vera* (L.) BURM. fil. (in Turkish “Sarisabir”) were collected from Kale (Demre) in Antalya, identified by N. Sütülpinar (May 1993; a voucher specimen was deposited in the Herbarium of Istanbul University, Faculty of Pharmacy, ISTE No. 65118), planted and cultivated in the greenhouse of the Faculty of Pharmacy. Fresh leaves of this cultivated plant were used in this study.

**Preparation of the Samples** *A. vera* leaves (6 big leaves) were weighed, washed and cut in the middle, the gel was separated by scratching with a spoon.

*Aloe vera* Leaf Pulp Extract: The leaf pulp was cut into small pieces (514 g) and homogenized with phosphate buffered saline (PBS; pH 7; 600 ml) by means of a Moulinex Masterchef blender. The extract was kept at 4°C overnight,

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then filtered through cloth and the filtrate centrifuged at 20000 rpm for 30 min at 4 °C in a refrigerated centrifuge (Cryofuge 20-3 Heraeus-Christ). The green pellet was discarded and the clear yellow supernatant was taken and lyophilized (Labconco apparatus). Thus, 29 g of *A. vera* leaf pulp extract was obtained. The extract used in the experiments (7.5%) was prepared by dissolving the powder in PBS and mixing it thoroughly via magnetic stirrer.

*Aloe vera* Leaf Gel Extract: The gel (400 g = 2.5 g dry matter) was homogenized in a Waring blender, then diluted with an equal volume of PBS and homogenized for a second time. The extract was kept at 4 °C overnight, then filtered through cloth. The clear filtrate was kept at −20 °C in small portions.

Glibenclamide Suspension: Glibenclamide (5 mg) was suspended in 21 ml PBS; 4 ml propylene glycol was added and the mixture was kept in an ultrasonic water bath (47.6 kHz) for 45 min until a homogenous suspension was obtained.

**Animals and Treatment.** Type-II Diabetic Model Wis-tar pups were injected intraperitoneally on day 2 after birth with STZ (n0-STZ rats), 100 mg/kg, freshly dissolved in cold citrate buffer (1 mM, pH 4.5) according to Bonner-Weir et al. This model of STZ-induced diabetes was reported by Portha *et al.* as potentially appropriate for investigations in diabetes pharmacotherapy. The animals were controlled for the occurrence of diabetes after 6 weeks and the diabetics (fasting blood glucose levels 104—170 mg/dl; mean 137 mg/dl, also in accordance with type-II diabetic model) were administered the above extracts orally each day for 15 d, by mixing it thoroughly with an equal volume of PBS and homogenized for a second time. The extract was kept at 4 °C overnight, then filtered through cloth. The clear filtrate was kept at −20 °C in small portions.

**Animal Groups** Group I: Healthy (non-diabetic; control) rats (5 animals) were kept under the same conditions as diabetic rats. Type-II diabetic rats were separated into 4 groups of 5—10 animals. Each group was given a sample as follows: Group II (untreated diabetic control): PBS (6 ml/kg), Group III: *A. vera* leaf pulp extract (500 mg/kg), Group IV: *A. vera* leaf gel extract (10 ml = 63 mg/kg), Group V: Gliben-clamide (1 mg/kg).

**Administration of Samples** Each group of animals was administered the above extracts orally each day for 15 d, by means of a catheter under mild ether anesthesia. The animals were sacrificed on the 15th day. Blood was taken by cardiac puncture, sera were separated for biochemical analysis. Liver tissues were taken for histological evaluation and biochemical assays.

**Histological Evaluation** The livers of healthy (non-diabetic) animals had a normal histological appearance (Fig. 1A). In the hepatocytes of the diabetic control group, excess vacuolization and granular appearance in the cytoplasm in the periphery of the nucleus, picnotic nuclei, dilations in the sinusoids, the rupture in the central vein and moderate hyperemia were observed. An increase in the degeneration was observed from central veins to portal areas (Fig. 1B). In the hepatocytes of the diabetic group given glibenclamide and *Aloe* extracts, in comparison to the diabetic control group however, it was noticed that the degenerative results were partly decreased in some animals and that the protective effect in tissues of these extract-administered diabetic groups was much greater than in the diabetic group given *Aloe* pulp, although there were individual differences (Figs. 1C—E).

**Biochemical Evaluation** According to Table 1, in liver tissues GSH levels did not change significantly in the PBS administered diabetic group compared to the healthy group. In contrast, these levels increased significantly with the treatment of *Aloe* pulp and gel extracts and glibenclamide in comparison to the diabetic control group (*p* < 0.001 for all groups). In diabetic controls, NEG values were significantly increased in comparison to healthy rats (*p* = Dunnett’s test) < 0.001). Compared to the diabetic control group, NEG values were decreased in groups given *Aloe* gel extract and glibenclamide (*p* < 0.001) but increased in the group given *Aloe* pulp extract. LPO products were significantly increased in the diabetic group given PBS compared to the healthy group (*p* = Dunnett’s test) < 0.001). *Aloe* pulp extract and glibenclamide significantly reduced LPO levels in comparison to diabetic controls (*p* = 0.05) whereas the decrease with *Aloe* gel extract was found insignificant (Table 1).

Serum ALP and ALT activities were significantly increased in type-II diabetic rats, compared to the healthy group (*p* = Dunnett’s test) < 0.001). Serum ALP activity was significantly reduced in groups given *Aloe* gel and glibenclamide (*p* < 0.05) but increased in the group given *Aloe* pulp in comparison to the diabetic control group. A significant decrease was found for ALT for the groups given *Aloe* leaf pulp and gel extracts (*p* < 0.001), whereas glibenclamide did not effect ALT levels in comparison to the diabetic control group given PBS. (Table 2).
DISCUSSION

In recent years, various plant extracts have been claimed to be useful for the cure of diabetes mellitus but few of them were tested for their effects on tissues of diabetic animals. Acute treatment with Aloe leaf pulp resulted in a 30% and 34% decrease in blood sugar levels of STZ-diabetic rats, after 2 and 3 h of administration of the extract respectively, whereas chronic treatment with the same extract leads to 7% decrease in blood glucose on the 7th day of administration. On the other hand, 11% and 14% reductions in blood glucose levels were observed 3 and 4 h after administration of Aloe leaf gel in acute studies while only 3% and 9% decrease in blood sugar was seen in chronic studies on the 7th and 14th days, respectively, after oral administration of the gel extract; under the same conditions 14% and 13% reductions were observed with glibenclamide. The present study was undertaken in order to examine morphologically and biochemically whether these Aloe extracts have a beneficial effect on liver tissue of type-II diabetic rats.

Fig. 1. Light Microscopic Photographs of the Livers of Rats in All Groups
(A) Liver of healthy rat (control). (B) Liver of type-II diabetic rat (control group given PBS). [Vacuolization (→), picnotic nuclei (▲), granular appearance in the cytoplasm (→), hyperemia (○), an increase in the degeneration to portal areas from central veins (→→)]. (C) Liver of diabetic rat given glibenclamide. (D) Liver of diabetic rat given Aloe gel extract. (E) Liver of diabetic rat given Aloe pulp extract (Masson ×320).
Aloe vera leaf gel extract has a protective effect comparable to aloeeresin derivatives recently reported.\(^{11,12}\) The authors wish to thank Prof. Dr. Nurhayat Sütlüpinar for her generous assistance. This work was supported by the Research Fund of Istanbul University. Project number: O-890/01122000.

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Table 1. Glutathione (GSH), Non-enzymatic Glycosylation (NEG), Lipid Peroxidation (LPO) Values for All Groups in Liver Tissues

<table>
<thead>
<tr>
<th>Group</th>
<th>GSH (nmol/mg protein)*</th>
<th>NEG (nmol fructose/mg protein)*</th>
<th>LPO (nmol MDA/mg protein)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Healthy (control)</td>
<td>18.64±0.12</td>
<td>2.62±0.26</td>
<td>1.01±0.26</td>
</tr>
<tr>
<td>II. Untreated diabetic (PBS control)</td>
<td>17.38±1.97</td>
<td>11.72±0.46((^{a}))</td>
<td>1.33±0.03((^{b}))</td>
</tr>
<tr>
<td>III. Diabetic + Aloe pulp</td>
<td>27.12±1.31((^{a}))</td>
<td>15.38±0.27((^{a}))</td>
<td>1.09±0.05((^{a}))</td>
</tr>
<tr>
<td>IV. Diabetic + Aloe gel</td>
<td>23.58±2.74((^{b}))</td>
<td>10.94±0.50((^{c}))</td>
<td>1.16±0.11</td>
</tr>
<tr>
<td>V. Diabetic + glibenclamide</td>
<td>25.09±1.85((^{b}))</td>
<td>9.38±0.19((^{c}))</td>
<td>1.10±0.04((^{c}))</td>
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*Mean±S.D.  a) p<0.001 compared to untreated diabetic control. b) p<0.001 compared to healthy control. c) p<0.05 compared to untreated diabetic control.

Table 2. Serum Alkaline Phosphatase (ALP), and Alanine (ALT) Transaminase Levels for All Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>ALP (U/l)*</th>
<th>ALT (U/l)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Healthy (control)</td>
<td>84.37±2.13</td>
<td>50.58±1.08</td>
</tr>
<tr>
<td>II. Untreated diabetic (PBS control)</td>
<td>160.90±4.76</td>
<td>78.67±2.61</td>
</tr>
<tr>
<td>III. Diabetic + Aloe pulp</td>
<td>201.50±10.92</td>
<td>59.42±3.94</td>
</tr>
<tr>
<td>IV. Diabetic + Aloe gel</td>
<td>118.89±10.92</td>
<td>60.75±7.50</td>
</tr>
<tr>
<td>V. Diabetic + glibenclamide</td>
<td>141.90±6.87</td>
<td>86.15±5.65</td>
</tr>
</tbody>
</table>

*Mean±S.D.  a) p<0.001 compared to healthy control. b) p<0.01 compared to untreated diabetic control. c) p<0.05 compared to untreated diabetic control. d) p<0.001 compared to untreated diabetic control.

The liver damage caused by diabetes is probably due to lipid peroxidation subsequent to free radical production. The increase in liver tissue LPO product levels in the diabetic control group compared to healthy animals (Table 1) is in agreement with the well-known fact that tissues of diabetic animals exhibit increased oxidative stress and disturbances in antioxidant defense compared to normal controls.\(^{38,39}\) This could be due to STZ treatment which generally induces oxidative predominance in tissues by generating nitrogen monoxide (NO).\(^{40}\) Aloe leaf pulp extract as well as glibenclamide have a beneficial effect on liver by lowering LPO levels (Table 1). This antioxidant effect may be due to the aloeeresin derivatives recently reported.\(^{11,12}\)

Common biochemical markers of liver damage are the increase in activity of some enzymes like ALT and ALP in the blood. The injection of total glycoside of Aloe vera var. chinesis was found to be effective in lowering the elevated ALT in experimentally induced liver damage.\(^{36}\) Aloe-emodin was also reported to have a protective effect on liver injury.\(^{41}\) In another study, fresh leaf pulp extract of Aloe vera caused a decrease in malondialdehyde (MDA) formation in liver, suggesting the plant’s role in the protection of oxidant induced cellular damage.\(^{42}\) In the examination of the enzymes characteristic for liver function, in the diabetic groups given Aloe pulp and gel extracts a decrease in serum ALT, in contrast to an increase in the group given glibenclamide (Table 2) shows that Aloe extracts are more beneficial for liver than this latter known hypoglycemic drug. Though ALP is a marker for different metabolic functions, which could also have been impaired by diabetes, Aloe pulp extract could have decreased ALT which is a mainly hepatic enzyme, but increased ALP. The fact that the activity of ALP is increased by Aloe leaf pulp extract but decreased by Aloe gel extract, tend to propose the use of Aloe gel in preference to Aloe pulp extract (Table 2).

It is concluded that, probably due to its antioxidant effects, Aloe leaf gel extract has a protective effect comparable to glibenclamide against hepatotoxicity produced by diabetes, and that Aloe gel could have a beneficial effect on liver if used as a hypoglycemic agent in the treatment of type-II diabetes.

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for providing and identifying the plant.

REFERENCES